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From: STIC-Biotech/ChemLib
Sent: Friday, July 18, 2003 8:33 AM
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From: Mayes, Laurie
Sent: Thursday, July 17, 2003 6:09 PM
To: STIC-Biotech/ChemLib
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Please send me a copy ~~of the~~ following:

Activation of factor VIII by thrombin increases its affinity for binding to phospholipid membranes.
AUTHOR: Saenko E L; Yakhyaev A V; Scandella D; Greco N J
JOURNAL: Blood 92 (10 SUPPL. 1 PART 1-2):p41A Nov. 15, 1998

Title: Role of the low density lipoprotein-related protein receptor in mediation of catabolism of coagulation factor VIII
Author(s): Saenko EL; Yakhyaev AV; Mikhailenko I; Strickland DK; Sarafanov AG
Journal: THROMBOSIS AND HAEMOSTASIS, 1999, S (AUG), P2-2

Thank you,

Laurie Mayes; AU 1653; 605-1208
CM1 10A16; Mailbox CM1 9b01

Abstract# 160 Poster Board#/Session: 160-I

ACTIVATION OF FACTOR VIII BY THROMBIN INCREASES ITS AFFINITY FOR BINDING TO PHOSPHOLIPID MEMBRANES. E.L. Saenko, A.V. Yakhyaev*, D. Scandella, N.J. Greco*. *Holland Laboratory, American Red Cross, Rockville, MD, USA.*

Membrane-bound thrombin-activated factor VIII (fVIIIa) functions as a cofactor for factor IXa in the factor Xase complex. We previously demonstrated that a conformational change occurs within C2 domain upon removal of the acidic region of the LCh during activation by thrombin. Since the site involved in fVIII binding to phosphatidylserine (PS)-containing membranes was localized to the C2 domain, we hypothesized that the conformational change could also affect fVIIIa affinity for phospholipid. We used a surface plasmon resonance (SPR) for detection of interaction of fVIII and its derivatives with immobilized small intact phospholipid vesicles. SPR permits a real time registration of association and dissociation processes and subsequent determination of the values of k_{on}, k_{off} and K_d. It was found that binding of heterotrimeric fVIIIa (A1/A2/A3-C1-C2) to synthetic vesicles with a physiologic content of 4% PS, 76% phosphatidylcholine (PC) and 20% phosphatidylethanolamine (PE) occurs with a 10-fold higher affinity than that of fVIII. The increased affinity of fVIIIa for PS-containing membranes resulted from the reduced k_{off} for fVIIIa dissociation from the PSPCPE (4/76/20) vesicles compared to that of fVIII. The k_{off} values for fVIII and fVIIIa are 1.7×10^{-3} s^{-1} and 2.3×10^{-4} s^{-1}, respectively, and the corresponding K_ds are 7.4 nM and 0.65 nM. Similar affinities determined for interaction of the A3-C1-C2, A3-C1-C2/heavy chain (HCh), and A1/A2/A3-C1-C2 with PS-containing membranes demonstrate that removal of the LCh acidic region by thrombin is responsible for these increased affinities of fVIIIa and its derivatives. Similar kinetic parameters of fVIII and its LCh and C2 domain for binding to PS-containing membranes and to activated platelets indicated that the C2 domain is entirely responsible for the interaction of fVIII with membranes. We conclude that the increased fVIIIa affinity for PS-containing membranes is a result of conformational change(s) within the C2 domain upon removal of the acidic region of the LCh. This conclusion is based on the finding that binding of the monoclonal antibody ESH8 to the C2 domain, which is known to prevent this conformational transition, resulted in fVIIIa binding to PSPCPE vesicles (4/76/20) with a lower affinity similar to that of fVIII. In addition, stabilization of the low affinity binding conformation of the C2 domain of fVIIIa by this antibody led to an inhibition of the formation of functional membrane-bound factor VIIIa-factor IXa complex.

Abstract# 161 Poster Board#/Session: 161-I

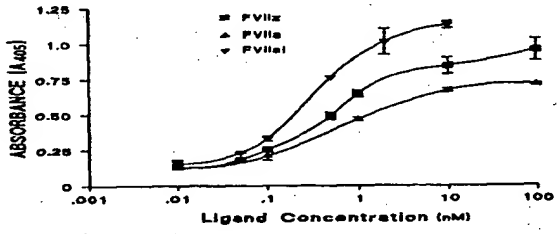
PHOSPHOLIPID-BOUND TISSUE FACTOR MODULATES BOTH THROMBIN GENERATION AND ACTIVATED PROTEIN C-MEDIATED FACTOR V_a INACTIVATION. K. Varadi*, J. Siekmann*, P.L. Turecek*, H.P. Schwarz*, V.J. Marder. *Baxter/Hyland-Immuno, Vienna, Austria; Vascular Medicine Unit, University of Rochester School of Medicine & Dentistry, Rochester, NY, USA.*

Hemostasis can be initiated by tissue factor (TF) exposure on cellular phospholipid (PL) membranes, leading to thrombin generation in a concentration-dependent manner. A counterbalancing process of coagulation inhibition involves thrombomodulin (TM) binding of thrombin, which in turn activates the protein C pathway and inactivates factors V_a and $VIII_a$. This report evaluates the potential for TF to not only induce thrombin generation but also to modulate (downregulated) protein C-mediated factor V_a inactivation on PL surfaces. A new assay system is developed for simultaneous measurement of thrombin and activated protein C (APC) generation in defibrinated plasma induced by large unilamellar vesicles (LUV) complexed with full-length recombinant TF in the presence of truncated recombinant TM. A series of LUV:TF complexes prepared by a free-thaw technique had distinct absolute and relative amounts of TF and PL, as quantitatively measured by direct assay. When assessed in our novel system, there was an expected strong correlation between TF concentration and the initial rate of thrombin generation. Below an initial thrombin generation rate of 100 nM/min, maximal thrombin concentration was decreased in the presence of TM ("feedback inhibition"), but thrombin generation above 100 nM/min prevented feedback inhibition, even though maximal APC concentrations were generated. Using the same LUV:TF surfaces to measure factor V_a inactivation by APC, we found a striking inverse correlation of TF spatial density (but not TF concentration) with factor V_a inactivation by APC. Thus, the data indicate that TF not only promotes thrombin generation but also impairs APC inactivation of factor V_a on PL surfaces. We propose a heretofore unrecognized hemostatic effect of TF, namely, a surface density based competitive inhibition of the anticoagulant effect of APC. In this model, high concentrations and surface density of TF exert complementary effects by promoting procoagulant and inhibiting anticoagulant mechanisms, thereby maximizing hemostasis after vascular injury.

Abstract# 162 Poster Board#/Session: 162-I

BOTH ACTIVATION AND ACTIVE SITE OCCUPATION OF HUMAN FACTOR VII ALTER CONFORMATION OF THE FIRST EGF-LIKE DOMAIN. B.J.N. Leonard*, B.J. Clarke, R. Kelley*, S. Sridhara*, F.A. Ofosu, M.A. Blajchman. *McMaster University, Hamilton, Ontario, Canada.*

Coagulation is initiated upon the interaction of cell surface tissue factor (TF) with circulating FVII. The EGF-1 domain of FVII is crucial for the binding of FVII to TF. However, investigations into the interaction of the EGF-1 domain of FVII with TF have been difficult to interpret, due to the multiplicity of TF binding sites associated with the FVII molecule. To better understand this interaction, the conformation-sensitive FVII EGF-1-specific murine monoclonal antibody (MoAb) 231-7 was used to investigate the conformational ramifications of zymogen FVII activation and FVIIa active site occupation (FVIIai). Initial analysis, using ELISA techniques, demonstrated that the affinity of MoAb 231-7 is highly sensitive to molecular perturbations of FVII related to the oxidation and conformational state of the EGF-1 epitope. Analysis of direct binding of FVII zymogen, FVIIa and FVIIai molecules to solid phase bound MoAb 231-7 showed distinctly different binding characteristics for each molecule (see Fig). The affinity of FVII for the MoAb was **decreased** upon activation of FVII to FVIIa, indicating a conformational change in the 231-7 epitope. The affinity of FVIIa for MoAb 231-7 was **increased** upon incorporation of the active site inhibitor FPRCK, indicating structural alterations to the EGF-1 region of a different nature than that produced by FVII activation. Determination of the K_Ds using a homogenous solution phase assay indicated the affinity of MoAb 231-7 for FVII zymogen was approximately 10X greater than to FVIIa, confirming the initial observations. These data provide the first direct evidence that conformational modifications in the region of the EGF-1 domain of FVII occur upon zymogen activation and active site occupation. These structural modifications probably affect the nature of the interaction of EGF-1 with TF, and indicate possible involvement of this region in the allosteric regulation of FVII biological activity.



Abstract# 163 Poster Board#/Session: 163-I

PROTEOLYSIS OF BLOOD CLOTTING FACTOR VIII BY THE FACTOR VIIa-TISSUE FACTOR COMPLEX. P.F. Neuenschwander, J.H. Morrissey. *Oklahoma Medical Research Foundation, Cardiovascular Biology Research Program, Oklahoma City, OK, USA.*

Activation of factor VIII (fVIII) to fVIIIa occurs via limited proteolysis (at Arg_{372}, Arg_{740} and Arg_{1689}) by thrombin or fXa. The resultant active fVIIIa consists of three non-covalently associated subunits (the A1 and A2 subunits (50 kDa and 43 kDa) derived from the heavy chain, and the A3-C1-C2 subunit (73 kDa) derived from the light chain) that can bind to fIXa to form the intrinsic fX-activating complex. Active fVIIIa is unstable, however, and rapidly dissociates leaving it susceptible to further proteolysis at Arg_{336} and Arg_{562} by thrombin, fXa, and activated protein C to generate inactive fVIIIai. We now find that the fVIIa-tissue factor complex (fVIIa-TF; the trigger of coagulation with known restricted substrate specificity) also catalyzes limited proteolysis of fVIII. Cleavage of fVIII (25 nM) by fVIIa-TF (10 nM) was monitored using SDS-PAGE with either silver staining or Western blotting using a polyclonal antibody to fVIII or an anti-A2 domain antibody. Proteolysis was seen within 1 min and produced two fragments (48 kDa and 43 kDa) recognized by the anti-A2 domain antibody. In contrast, only the 43 kDa fragment was observed with thrombin as the activator. Longer time courses with fVIIa-TF indicated slow conversion of the 48 kDa fragment to 43 kDa, followed by further degradation into at least two fragments. *N*-terminal sequencing of the major fragments showed that fVIIa-TF can cleave fVIII at all five above cleavage sites. However, cleavage at the "inactivation" site Arg_{336} occurs first or concomitant with cleavage at the "activation" site Arg_{372}. While light chain cleavage at Arg_{1689} was observed, the majority of the light chain remained uncleaved after 17 h. Consistent with these results, no increase in clotting activity was observed over unactivated fVIII when compared to thrombin-activated fVIII. Thus, heavy chain cleavage of fVIII by fVIIa-TF seems to produce an unactivatable fVIII species without proceeding through an active intermediate. While the *in vivo* significance of this proteolysis is unknown, we hypothesize that it may constitute an alternative pathway to regulate fX activation under certain conditions. In addition, the ability of fVIIa-TF to cleave fVIII at these five sites greatly expands the known substrate sequences recognized by the highly specific fVIIa-TF complex.